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RESEARCH ARTICLE

SHORT TERM PRESERVATION OF GOAT SKIN WITH INDIGENOUS *AZADIRACHTA INDICA* LEAF TO REDUCE POLLUTION LOAD IN LEATHER PROCESSING

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ABSTRACT

The most common method of preserving of raw hides/skins is curing with sodium chloride. Soaking is the first physiochemical operation in tannery, where huge amount of curing salt is released to environment and increased salinity of the wastewater in the form of total dissolved solids (TDS). Chloride is highly soluble and stable that is not affected by the effluent treatment plant. In this study, goat skin was preserved using indigenous *Azadirachta Indica* (neem) leaf paste with potassium chloride (KCl) to reduce the pollution load in the wastewater. The efficacy of the alternative system was assessed by monitoring the parameters *e.g.* total viable count (TVC), total coliform count (TCC), total *Staphylococcus* count (TSC), hair slip, odor, isolated bacterial count and loss of moisture content. Results revealed that preservation of goat skin using *Azadirachta Indica* leaf paste with 10% potassium chloride (KCl) was effectively preserved the goat skin for the span of 30 days.

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INTRODUCTION

Animal skins, the byproduct of the meat industry are used as the basic raw materials in tanning industry. In living animal, hide/skin contains bacteria and microorganism held in control by the metabolic defenses of animal. In raw hide/skin contains water 60-70% and protein 25-30% (Rao and Henrickson, 1983). The raw hide/skin is vulnerable for bacterial attack, which starts within 5-6 hours after the animal death (Edward *et al.,* 2008). Thorstensen reported that the process of decay starts immediately after killing the animal (Thorstensen, 1993). The raw hide/skin has to be preserved quickly to protect it from the microorganisms during storage and transport until it is converted into leather, otherwise it will begin to decay. Preservation of hide/skin should ideally begin instantly after flaying and should never be late overnight (Leach and Wilson, 2009). In the tropical country like Bangladesh and India, the most popular practice of using common salt (NaCl) in preservation of hide/skin due to having dehydration property.

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The hide/skin is preserved by application of sodium chloride at a concentration of 40-50%. The common salt has dual functions as curing agent: i) dehydrating ability and ii) bacteriostatic effects (Sivabalan and Jayanthi, 2009). The preserved hide/skin is subsequently process in tanneries generate a large quantities of total dissolved solids (TDS) which is not affected by the effluent treatment plant (Kanagaraj *et al.,* 2005a). In tannery, the first unit operation is soaking where releases nearly 55% of the total dissolved solids (TDS) of the entire leather processing (Ludvik, 1996). Chlorides are highly soluble and stable those are not affected by effluent treatment as a result, it remains as a burden to the environment. Per ton of leather processing nearly 40,000 liters of effluent are being generated which contributing about 350-450 Kg of salt as TDS (Preethi *et al.,* 2006).

The high amount of salt contained in the effluent when used for irrigation purpose which will be increased the salinity thus reducing the fertility of soil. To date there is no technology available for treating the effluent contain high amount of common salt (Kanagaraj and Basu, 2002). Many researchers and scientists have been investigated the preservation of hide/skin in various methods. The chemicals or reagents such

as sodium sulphate (Vankar and Dwivedi, 2009), sodium meta-bisulphite with acetic acid (Kanagaraj *et al.,* 2005b), silica gel (Kanagaraj *et al.,* 2001), potassium chloride (Bailey and Gosselin, 1996), boric acid (Hughes, 1974), neem (*Azadirachta Indica*) oil with sodium chloride (Khan *et al.,* 2005), soda ash (Rao and Henrickson, 1983), formaldehyde (Sharphouse, 1978), biocidal methods such as use of benzalkonium chloride (Cordon *et al.,* 1964), antibiotics like aureomycin and terramycin, tetracycline (Berwick *et al.,* 1996) were found as the curing agents. Similarly, physical methods of preservation like controlled drying in a drying chamber (Waters *et al.,* 1997), radiation curing by using gamma rays and electron beam (Bailey, 1997) for preserving the hide/skin were also found as curing methods. Here an approach has been taken in view of short term preservation of goat skin using *Azadirachta Indica* (Neem) leave paste with potassium chloride (KCl) for the reduction of pollution load in leather processing. The efficacy of the alternative system was assessed by monitoring the parameters *e.g.* total viable count **(**TVC), total coliform count (TCC), total *Staphylococcus* count (TSC), hair slip, odor, isolated bacterial count and loss of weight.

MATERIALS AND METHODS

Sample collection

A total number of seven raw goat skins were collected from the local market at Hazaribagh, Dhaka after the usual traditional method of flaying. The skins were brought to the laboratory without controlling temperature and humidity. After removal of extra meat and fats, the skins were washed with plain water to remove blood, dirt and other contaminants. *Azadirachta Indica* (Neem) leaves were collected from the local *Azadirachta Indica* (Neem) trees. The chemicals like sodium chloride, boric acid, zinc chloride, potassium chloride, bactericide, etc. were from the commercial grade and collected from the local market.

Methodology

The flesh side of the six pieces of goat skins were treated with *Azadirachta Indica* (Neem) leaves paste, common salt (NaCl) (commercial grade), boric acid (commercial grade), zinc chloride ($ZnCl_2$) (commercial grade), potassium chloride (KCl) (commercial grade), bactericide (Busan 40L, Buckman Lab.). The treated goat skins were kept on an open space in the leather workshop and periodically observed for assessment. The goat skins were treated with different curing agents is furnished in

Table I. Curing of goat skins treated with different curing agents

Sample ID	Curing agents	Storage time (days)
T0	No treatment, as control	03
T1	20% Neem leaves paste and 5% KCl	30
T2	25% Neem leaves paste and 10% KCl	30
T3	50% Salt, NaCl	30
T4	25% Salt and 4% Boric Acid	30
T5	30% Salt and 3% $ZnCl_2$	30
T6	Saturated salt solution and 0.1% Busan 40L	30

Bacteriological study

A 10 g sample was taken from each of the sample skins untreated and treated with various curing agent to identify the bacteria that contaminated the skins. The efficacy of curing agents to minimize bacterial load and the survivability of microbes were also studied (Rashid *et al.,* 2008). The skin portion 10 g of each was excised from skin samples using sterile instruments and transferred carefully to sterile containers for bacteriological analysis. A 1:10 diluted sample was prepared by adding 90 mL of sterilized normal saline to every individual raw and preserved sample and homogenized in stomacher at 230 rpm for 30s. Subsequently, using whirly mixture different serial dilutions were prepared ranging from 10^{-2} to 10^{-5}.

Determination of TVC, TCC and TSC

To determine TVC, 1 mL of each ten-fold dilution was transferred and spread on Petri dish containing Tryptic Soy Agar (OXOID) media using a sterile pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile plastic spreader. Separate sterile spreader was used for each plate. The plates were then kept in an incubator at 37^0C for 24-48 hours. Following incubation, plates exhibiting the colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the TVC. In order to determine the TCC, Chromocult Agar (SCHARLAU) plates were used. The procedures of sampling, dilution and streaking were similar to those followed in TVC of bacteria. Only in case of *staphylococcus* count, Mannitol Salt Agar (SCHARLAU) plates (Hardy diagnostics) were used and kept in an incubator at 30^0C. The calculation for TSC was similar to that of TVC.

Isolation and identification of bacteria from the skin samples

Isolation and identification of bacteria in the skin samples were based on the morphology (size, shape, arrangement and motility) and colony characteristics.

Isolation and identification of *Staphylococcus*: In view of isolation and identification of *Staphylococcus*, the samples were diluted, inoculated on Mannitol Salt Agar (SCHARLAU) plates and incubated at 30^0C for 24 hours. Mannitol salt agar is a selective and differential media for *Staphylococcus. Staphylococcus aureus* produced yellow colonies with yellow zones, whereas other *Staphylococci* produced small pink or red colonies with no color change to the media after 24 hours of incubation.

Isolation and identification of *Escherichia coli* (*E. coli*): With a view to isolation and identification of *Escherichia coli* (*E. coli*), the samples were diluted, inoculated on Sorbitol MacConkey agar (SCHARLAU) and incubated at 37^0C for 24 hours. Sorbitol MacConkey agar is a selective and differential media for enterohemorrhagic *E. coli*. Traditionally, MacConkey agar has been used to distinguish those bacteria that ferment lactose from those that do not. *E. coli* can typically ferment lactose. In sorbitol MacConkey agar, lactose is replaced by sorbitol. Non-pathogenic strains of *E. coli* ferment sorbitol to produce acid. Pathogenic *E. coli* cannot ferment sorbitol. Pathogenic *E. coli* produced off white colonies on Sorbitol MacConkey agar where Non-pathogen *E. coli* produced pink colonies on Sorbitol MacConkey agar after 24 hours of incubation.

Isolation and identification of *Bacillus sp*: For the isolation and identification of *Bacillus sp.*, the samples were diluted, inoculated on NaCl Glycine Kim Geopfert agar (SCHARLAU) and incubated at 30^0C for 24-48 hours. *Bacillus sp.* produced white colonies on NaCl Glycine Kim Geopfert agar with changing the color of media into pink after 24 hours of incubation.

RESULTS AND DISCUSSION

Population of bacteria in preserved skin

The population of bacteria in the cured skin is inserted in Table II. The numbers of *E. coli*, *Staphylococcus sp.* and *Bacillus sp.* present in the samples were quite remarkable. The *Staphylococcus sp.* will be high if the goat was affected by arthritis whereas *E. coli* count will be high while the disease like Colibacillosis/colisepticemia affected them during living. The skins collected from unhygienic carcass sites show high number *E. coli*, *Staphylococcus sp.* and *Bacillus sp.* bacterial count. Table II shows the TVC present in the samples those include all types of bacteria are quite high. TCC count includes coli forming bacteria and TSC includes total staphylococcus bacteria in the samples. The T1 and T2 gives higher TSC count than T3, T4, T5 and T6, which revealed that the salt tolerant organisms of the skin gradually adapted themselves and grow even at high salt concentrations.

waste products due to bacterial activity. The rest of the samples from T1 to T6 different curing agents were used and there was no discoloration of the skin or hair slip or any bad odor. The skin exhibiting hair slip had been discarded and no attempt was made to process.

Isolated bacteria in the skin

The isolated bacteria in raw and cured skin are shown in Table IV. The *Staphylococcus sp*. was observed alarming level in most of the samples. The presence of pathogenic bacteria *E. coli* was found in the sample T0 to T4. In sample T0 to T2 *E. coli* was fairly higher than in the samples T3 and T4. In sample T5 and T6, the level of *E. coli* was below the detection level. The pathogenic *Bacillus sp.* was found in sample T2 to T6 and in the sample T0 and T1 it was below the detection level. The occurrence of pathogenic bacteria *E. coli* and *Bacillus sp.* receive special attention since these organisms are responsible for causing hazards to public health. Both fresh and salted skins have been shown to be contaminated with *Bacillus sp., Micrococcus sp.* and *Staphylococcus sp.* but the greater danger of damage seems to be from the *Bacillus sp.* (Covington AD, 2011).

Percentage of weight loss of the cured skins

It has been realized from the Fig. 1 that the curing agent T2 evolved more moisture than the conventional curing agent

Table 2. Selected bacteria expressed in CFU/mL (CFU= Colony Forming Unit)

Bacterial count	Survivability of viable bacteria in cured skin						
	T0	T1	T2	T3	T4	T5	T6
	0 Days	30 Days	30 Days	30Days	30 Days	30 Days	30 Days
TVC	1.26×10^7	1.09×10^8	9.12×10^7	1.9×10^5	8.6×10^4	4.6×10^4	1.6×10^6
TCC	3.8×10^6	3.5×10^6	2.5×10^6	2.0×10^3	6.9×10^4	1.7×10^4	5.2×10^4
TSC	9.7×10^6	5.76×10^8	3.28×10^8	2.8×10^5	1.48×10^5	2.4×10^4	3.08×10^5

Table 3. Storage property of skin treated with various curing agents

Sample ID	Storage time (day)	Color changes	Hair slip	Foul odor
T0	03	Discolored	Full hair slip occurred	Pungent odor
T1	30	Nil	No	Nil
T2	30	Nil	No	Nil
T3	30	Nil	No	Nil
T4	30	Nil	No	Nil
T5	30	Nil	No	Nil
T6	30	Nil	No	Nil

Table 4. Isolated bacteria in the cured skins treated with various agents

Isolated bacteria	T0	T1	T2	T3	T4	T5	T6
Escherichia coli	6.2×10^6	5.0×10^5	1.0×10^7	3.7×10^3	2.24×10^4	ND*	ND*
Staphylococcus sp.	9.7×10^6	5.76×10^8	3.28×10^8	2.8×10^5	1.48×10^5	2.4×10^4	3.08×10^5
Bacillus sp.	ND*	ND*	8.5×10^5	4.0×10^2	1.1×10^4	3.0×10^3	2.5×10^4

ND*= Not Detected

Physical changes of the skin with selective curing agents

The changes of physical appearance of the skin with different curing agents during storage are inserted in Table III. The sample T0 was without any curing agent causes discoloration of the skin, fully hair slip with pungent odor. Hair slip is one of the most common sign of spoilage the skin. The pungent odor and the discoloration of spoiled skins were usually attributed to the (sodium chloride). The Table IV has also shown that the most detrimental bacteria for the skin, *Bacillus sp.* were present in all skin samples except the skin sample T1.

It is observed that sodium chloride held more moisture in the skin sample T3 (50% Salt) and T6 (Saturated salt solution and 0.1% Busan 40L) and lost less weight.

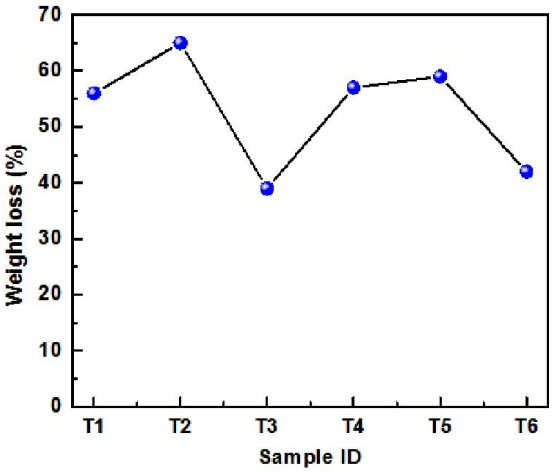

Fig. 1. Percentage of weight loss of the cured skins

This study was conducted during rainy season that was conducive for the bacterial growth due to high relatively humidity and temperature. It was found that the skin treated with sodium chloride absorbed moisture from atmosphere at about 30^0C temperature and 90% relative humidity. The storage temperature and relative humidity is very important factors for the skin treated with sodium chloride (T3).

Conclusion

The proposed short term preservation system using indigenous *Azadirachta Indica* leaf paste with potassium chloride was a cleaner approach towards overcoming the environmental constraints of using high concentration of salt in preservation of goat skins. The study reveals that *Azadirachta Indica* leaf paste with potassium chloride was effectively preserved the goat skin for 30 (thirty) days. This span of time is enough to handle and transfer the preserved goat skin to the tannery from any part of the country. The used *Azadirachta Indica* leaf with nutritious potassium chloride can be collected from the preserved goat skins before the first unit operation of leather processing called soaking and that can be used as good manure in agricultural cultivation.

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